# Investigation of a novel epiphytic cyanobacterium associated with reservoirs affected by avian vacuolar myelinopathy

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## Introduction
The recovery and conservation of bald eagles (*Haliaeetus leucocephalus*) has been contested due to a newly identified fatal bird disease: Avian Vacuolar Myelinopathy (AVM). Since the discovery of the disease in 1994, AVM has caused mortality in at least 100 bald eagles, thousands of American coots, and other various species of bird throughout the southeastern US.  AVM has been found in AR, TX, NC, SC, and GA. The cause of the disease has yet to be identified.

A strong association has been observed between the occurrence of AVM, *Hydrilla verticillata* (hydrilla), and a novel potentially toxic, epiphytic cyanobacterium on hydrilla.  The correlation has led to the hypothesis that this epiphyte is the source of the neurotoxin causing AVM. During 2001-2004, the Stigonematales species was present on the surface of hydrilla at every site where AVM had been diagnosed, but was absent or scarcely found in areas where AVM was not observed.

## Hypotheses
It is hypothesized that the proposed toxin of the cyanobacteria is bioaccumulated through the food chain from waterfowl (e.g. coots) ingesting the Stigonematales species growing on the hydrilla.  The goals of the study included; establishing a culture of the targeted Stigonematales species, expanding morphological descriptions of the species, determining gene sequence data from material collected in the field, and developing a Real Time-PCR assay specific to the cyanobacterium.

## Methods
A monoculture of the cyanobacterium was established on BG-11 medium at 27°C.  The 16S rRNA sequence identity was determined from environmental isolates using DGGE and then "ground-truthed" with culture isolates. The 16S rRNA sequence data were aligned with additional cyanobacteria sequences to determine designations for probe development, and to use in phylogenetic analysis.  Real-Time PCR assays were developed specific to the Stigonematales species.

## Results
The Stigonematales species has been cultured in order to aide in development of species identity, genetic research, feeding trials, and toxin analysis.  16S rRNA sequence data were aligned with additional cyanobacteria sequences to advance understanding of the species' phylogeny, and to lay groundwork for its formal description.  Phylogeny data confirmed that the species is in section V, order Stigonematales.  Phylogeny also inferred that the species is novel and most genetically similar to a *Stigonema* sp.  Based on sequence variability, a Real Time-PCR assay has been developed for rapid, specific detection of the Stigonematales species from environmental samples.

## Conclusion
The dominant epiphyte found on collected *Hydrilla* is an undescribed species of cyanobacterium in the order Stigonematales.  The genetic probe and taxonomic information produced by this study will help test the hypothetical link between these cyanobacteria and AVM, and therefore help guide decisions on managing hydrilla and other invasive macrophytes in AVM-affected waters.